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Immunology

Binding site for IgE of the human lymphocyte low-affinity Fc$_\epsilon$ receptor (Fc$_\epsilon$RII/CD23) is confined to the domain homologous with animal lectins

(IgE-binding factor/structure-function analysis/epitope mapping/mammalian expression vector)

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ABSTRACT The lymphocyte low-affinity receptor for IgE (Fc$_\epsilon$RII) is involved in two seemingly unrelated processes: (*i*) promotion of general B-cell growth and (*ii*) isotype-specific IgE synthesis. To characterize domains of Fc$_\epsilon$RII important for effector function, we have expressed Fc$_\epsilon$RII mutants in mammalian cells. The results show that the IgE-binding region of Fc$_\epsilon$RII corresponds almost exactly to a domain of 123 amino acid residues homologous with the carbohydrate-binding domain of C-type animal lectins. With the recent demonstration that Fc$_\epsilon$RII binds to IgE independently of any lectin-like activity [Vercelli, D., Helm, B., Marsh, P., Padlan, E., Geha, R. S. & Gould, H. (1989) *Nature (London)* 338, 649–651], it is now clear that, in this case, the lectin module has evolved to interact with a protein rather than a carbohydrate moiety. The epitopes of several independent monoclonal antibodies that inhibit the binding of IgE to Fc$_\epsilon$RII are clustered within the lectin-like domain. Some of these antibodies are also known to suppress, isotype-specifically, the interleukin 4-promoted IgE synthesis from peripheral blood mononuclear cells or the spontaneous synthesis of IgE by B cells isolated from atopic donors. The epitope of MHM6, an anti-Fc$_\epsilon$RII monoclonal antibody delivering an epitope-restricted growth-promoting effect on B cells, is also located within the lectin-like domain. Thus, the lectin module of Fc$_\epsilon$RII not only acts as a carbohydrate-independent, isotype-specific Fc receptor but may also participate in the general regulation of B-cell growth.

IgE is the class of immunoglobulin responsible for most allergic diseases (1) and is involved in immunity against parasites (2). The effector functions of IgE are brought about by binding to specific cell surface receptors. A high-affinity receptor (Fc$_\epsilon$RI) and a low-affinity receptor (Fc$_\epsilon$RII) for the Fc part of IgE have been described in the human system (3–6). Fc$_\epsilon$RI mediates effector release from mast cells and basophils in immediate-type hypersensitivity reactions. Fc$_\epsilon$RII, identical with the 45-kDa B-cell differentiation antigen CD23 (7, 8), is structurally distinct from the four-chain Fc$_\epsilon$RI molecule. Fc$_\epsilon$RII is expressed on many leukocytes and plays a role in isotype-specific processes such as regulation of IgE synthesis (6, 9, 10) and IgE-dependent cytotoxicity against parasites (2). Fc$_\epsilon$RII is also important in more general B-cell growth-regulatory pathways (5, 11–13). MHM6, an anti-Fc$_\epsilon$RII monoclonal antibody (mAb), has been shown to trigger the progression of activated B cells through the G$_1$ phase of the cell cycle (11, 12). Beyond its implication in a specific as well as in a more general B-cell regulation system, a role for Fc$_\epsilon$RII in cell adhesion has also been proposed (14, 15).

The cDNA for the human Fc$_\epsilon$RII has been cloned and functionally expressed in mammalian cells (16–18). It encodes a protein of 321 amino acid residues with an inverse membrane orientation; the N terminus is cytoplasmic and the C terminus is exposed at the cell exterior. The C-terminal half of the extracellular domain is shed from the membrane as a 25-kDa molecule as a result of proteolytic cleavage. This soluble fragment has been termed an IgE-binding factor (IgE-BF). It is found complexed with IgE in the serum of atopic patients (7) and its level is highly elevated in patients with B-cell-derived chronic lymphocytic leukemia (19). Furthermore, T cells of patients infected with human immunodeficiency virus type 1 are induced to produce IgE-BF (20).

The N terminus of Fc$_\epsilon$RII-derived IgE-BF starts at amino acids 148–150 of Fc$_\epsilon$RII (16–18). The middle region of this IgE-BF shows a marked degree of homology with animal lectins (21, 22), nonenzymatic proteins that bind selectively to specific carbohydrate structures (23). Recently, it became evident that there exists in the hematopoietic system a family of adhesion receptors, including Mel-14, ELAM-1, and GMP-140, with homology to the lectin domain of Fc$_\epsilon$RII (24). These molecules control lymphocyte migration, probably through carbohydrate-dependent recognition events.

The present experiments were undertaken to delimit the IgE-binding domain of Fc$_\epsilon$RII. Further, we have mapped the epitopes of several anti-Fc$_\epsilon$RII mAbs that have been reported to influence either the isotype-specific or the B-cell growth-promoting processes.

MATERIALS AND METHODS

Assembly of Expression Plasmid pCAL5mβDhfr for Mammalian Cells. Plasmid pSVneo2911 (25) was used to prepare a *Sal* I–*Eco*RI DNA fragment containing the ampicillin-resistance, tetracycline-resistance, and dihydrofolate reductase (Dhfr) genes. The murine cytomegalovirus (MCMV) immediate-early gene enhancer and promoter, used to drive Fc$_\epsilon$RII cDNA expression, are contained in the 540-base-pair (bp) *Acc* I–*Hin*dIII fragment of the simian virus 40–MCMV recombinant virus P1 (26). The 1.26-kilobase (kb) Fc$_\epsilon$RII cDNA insert was prepared by digesting plasmid pSVd-ER (17) partially with *Hin*dIII and completely with *Bam*HI. The 3′ half of the rabbit β-globin gene was derived from plasmid pUβ (27). Its 1.2-kb *Bam*HI–*Sal* I fragment contains the large β-globin intron and the polyadenylylation signal. All these cartridges were combined to construct the expression plasmid pCAL5mβDhfr (Fig. 1). Incompatible restriction cleavage sites were filled in with Klenow enzyme and joined by blunt-end ligation.

Construction of Fc$_\epsilon$RII Deletion Mutants. The *Sal* I–*Eco*RI DNA fragment from plasmid pSVd-ER (17) containing the

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Abbreviations: Fc$_\epsilon$RII, lymphocyte low-affinity receptor for IgE; mAb, monoclonal antibody(ies); pAb, polyclonal antibodies; Dhfr, dihydrofolate reductase; IgE-BF, IgE-binding factor(s); MCMV, murine cytomegalovirus; BCGF, B-cell growth factor.

EXHIBIT B

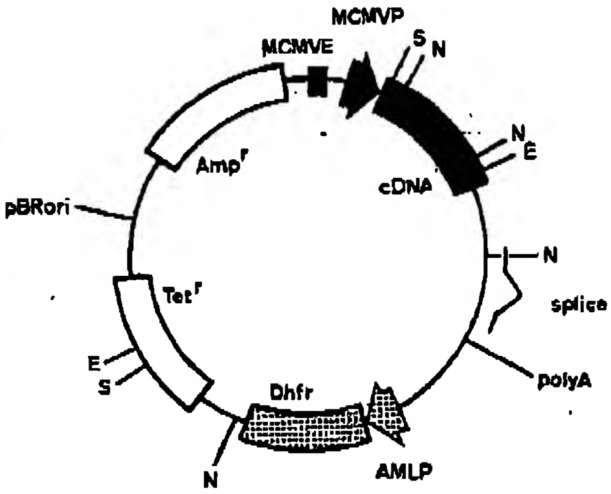

FIG. 1. Mammalian expression vector pCAL5mβDhfr. The region encoding various Fc$_\epsilon$RII constructs is shown by a heavy black bar. cDNA expression is driven by an enhancer/promoter unit (MCMVE/MCMVP) of the MCMV (black box and arrow). The Dhfr coding sequence (stippled bar) is under control of the adenovirus major late promoter (AMLP; stippled arrow). The genes for ampicillin (Amp^r) and tetracycline (Tet^r) resistance are shown as open bars. Restriction sites indicated were used for construction of Fc$_\epsilon$RII mutants (N, *Nco* I; E, *Eag* I; S, *Sal* I). In addition, the rabbit β-globin polyadenylylation (polyA) and splice sites as well as the bacterial pBR322 replication origin (pBRori) are shown.

entire coding region of Fc$_\epsilon$RII was inserted into the polylinker of Bluescript M13 phagemid SK− (Stratagene). After infection with helper phages, single-stranded phage DNA carrying the cDNA insert was purified and used as template for mutagenesis. Oligonucleotide-directed *in vitro* mutagenesis (Amersham kit) employed methods recommended by the manufacturer, except that the exonuclease III treatment to remove the nonmutated strand was shortened to 20 min. All oligonucleotides used in the mutagenesis reaction were purified by PAGE prior to use. C-terminal deletion mutants were constructed by inserting the stop codon TGA into the reading frame. Internal deletions were generated by looping out the desired cDNA region in the mutagenesis reaction. To allow rapid screening, mutants were designed so as to introduce a novel restriction site into the cDNA without altering the amino acid sequence. Plasmids bearing each mutation were cleaved with *Sal* I and *Eag* I, which remove a cDNA fragment covering the base changes introduced. This fragment was ligated to two other DNA fragments (*Sal* I–*Sal* I, containing the ampicillin-resistance gene, and *Sal* I–*Eag* I, containing the Dhfr gene cassette) to restore the mammalian expression vector pCAL5mβDhfr (Fig. 1). The double mutant Δ134–160/X288 (Fig. 2) was obtained by combining the two corresponding deletion mutants. Control plasmid Δ1–298 (Fig. 2) was constructed by removing the *Nco* I–*Nco* I cDNA fragment of pCAL5mβDhfr. Sequence analysis (Sequenase kit, United States Biochemical) of the mutations was performed on the double-stranded expression plasmids with the use of synthetic oligonucleotide primers.

Antibodies Specific for Fc$_\epsilon$RII. We used a recombinant C-terminal Fc$_\epsilon$RII fragment (amino acids 119–321) produced in *Escherichia coli* to immunize rabbits. This protein elicited an immune response against native Fc$_\epsilon$RII. The polyclonal antibodies (pAb) were purified by standard methods. All the anti-Fc$_\epsilon$RII mAb used have been described: mAb135, mAb176, mAb168, and mAb64 (28); mAb25 (8); MHM6 (24); mAb3-5 (30).

Stable Transfectants of Fc$_\epsilon$RII cDNA Mutations. Transfection of Dhfr$^-$ Chinese hamster ovary (CHO) DUKX-B1 cells (31) was performed by the calcium phosphate technique as described (32). After 14 days of Dhfr selection, at least 50 resistant colonies were pooled for analysis. Expression of mutant Fc$_\epsilon$RII on the cytoplasmic membrane was demonstrated by incubation of glutaraldehyde-fixed cells with the Fc$_\epsilon$RII-specific pAb (1:2000 dilution) at 37°C for 1 hr and subsequent staining with a goat anti-rabbit antibody coupled to peroxidase (Bio-Rad). The same procedure was used for staining of cells with mAb (10 μg/ml), except that a goat anti-mouse (Bio-Rad) second antibody was used.

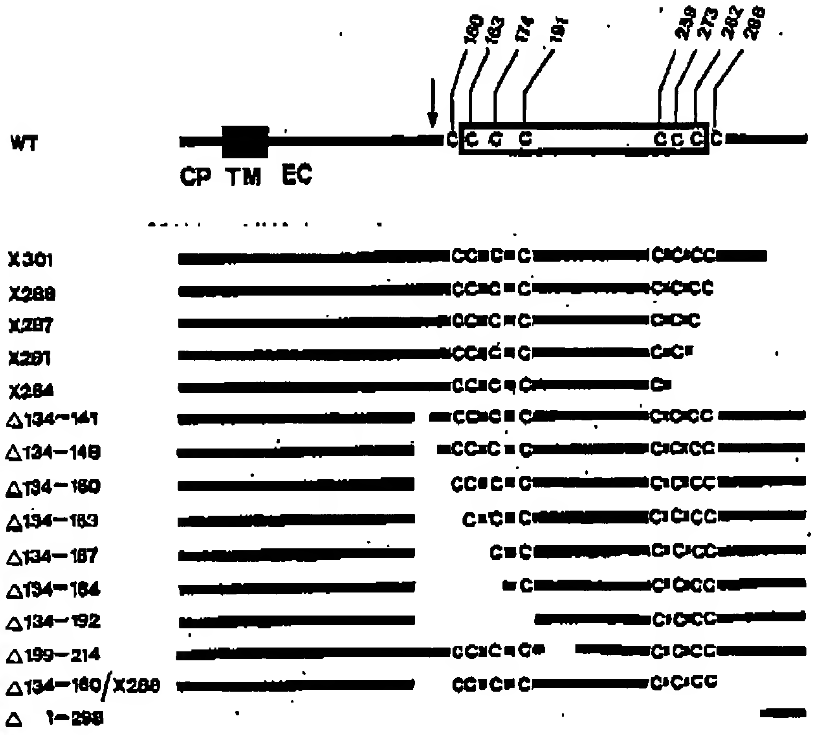

FIG. 2. C-terminal and internal deletions in the Fc$_\epsilon$RII cDNA. A schematic figure of the entire coding region (wild type, WT) is given at the top. The black box shows the transmembrane (TM) region; the N terminus is exposed to the cytoplasm (CP) and the C terminus is extracellular (EC). The open box represents the cysteine-rich domain homologous with animal lectins. The proteolytic cleavage site for the 25-kDa IgE-BF is indicated with an arrow. Cysteine residues (C) are numbered. The mutants are listed below. Black lines indicate those parts of the protein that are retained in a particular mutant. Deleted sequences are omitted. The name of each mutant is given at the left. Mutants were named as follows. Residues are numbered from the N terminus to the C terminus of the wild-type Fc$_\epsilon$RII. C-terminal truncation mutants (X) contain residues extending from the N terminus through the given amino acid number. Internal deletion mutants (Δ) lack the residues delineated by the numbers. Mutant Δ134–160/X288 encompasses exactly the region containing the 8 extracellular cysteines. A control expression vector, Δ1–298, lacks nearly the entire cDNA insert.

Immunoblot Procedures. Stably transformed CHO cells were harvested and washed in Dulbecco's phosphate-buffered saline without calcium and magnesium. Cellular extracts were obtained after homogenizing the cells in lysis buffer [50 mM Tris·HCl, pH 7.8/5 mM $MgCl_2$/1 mM $CaCl_2$/1% (vol/vol) Nonidet P-40/0.5% (wt/vol) phenylmethylsulfonyl fluoride] and removing the nuclei by sedimentation at 4000 × *g*. Detergent extracts corresponding to 10^6 cells were subjected to reducing (5% 2-mercaptoethanol in sample buffer) or nonreducing $NaDodSO_4$/12% PAGE (33). The proteins were electrophoretically transferred with a semidry blotter (Sartorius SM17556) to Immobilon membrane (Millipore). The membrane was blocked for 30 min with 50% goat serum in TBST (10 mM Tris·HCl, pH 8.0/150 mM NaCl/0.05% Tween-20/0.1% NaN_3). After overnight incubation with pAb (1:5000) or mAb (mAb135, -176 or -168 at 10 μg/ml; mAb64 at 2 μg/ml; or MHM6 as a 1:500 dilution of ascites fluid) diluted in blocking buffer, the membrane was rinsed in TBST and incubated for 30 min with alkaline phosphatase-labeled goat anti-rabbit or goat anti-mouse antibodies (Bio-Rad), respectively. The membrane was rinsed and developed with the substrates *p*-nitro blue tetrazolium chloride (330 μg/ml) and 5-bromo-4-chloro-3-indolyl phosphate toluidine (165 μg/ml) (Bio-Rad) in 100 mM Tris·HCl, pH 9.5/100 mM NaCl/5 mM $MgCl_2$.

Rosetting Assay. A 1:10 dilution of latex beads (Sigma LB-11, diameter 1.09 μm) was incubated with BSA-NIP (4-hydroxy-3-iodo-5-nitrophenylacetic acid coupled to bovine serum albumin, 0.3 mg/ml; ref. 34) in 15 mM sodium acetate (pH 5.0) for 1 hr. After two washes in phosphate-buffered saline, the beads were incubated for 1 hr in RPMI 1640 medium (GIBCO) supplemented with 15% fetal bovine serum. The beads were washed twice and resuspended in RPMI 1640 with 15% fetal bovine serum and specific anti-NIP IgE (20 μg/ml). After washing, the IgE-coated beads were sedimented onto stably transformed CHO cells (10^4 cells per well of a 96-well plate) by centrifugation at 200 × *g*. All these steps were done at room temperature. After 2 hr of incubation on ice, unbound beads were removed by gentle washing. The specificity of the rosetting was controlled by inhibition of the reaction with IgE (1 mg/ml) or Fc$_\epsilon$RII-specific pAb (1:5000 dilution).

RESULTS

Design of Fc$_\epsilon$RII Deletion Mutants. A striking feature of Fc$_\epsilon$RII is a region of 123 amino acid residues that shows a pronounced homology with the carbohydrate-binding domain of animal C-type lectins. This homology domain is completely contained within the 25-kDa IgE-BF released from the Fc$_\epsilon$RII by proteolytic cleavage (16–18). The IgE-BF encompasses 8 cysteines, of which 6 are part of the homology domain and the remaining 2 flank it very closely (21, 22). To delimit functional domains, in particular the one for IgE binding, we constructed internal and C-terminal deletion mutants that lack the cysteines in a progressive order (Fig. 2).

Expression of Fc$_\epsilon$RII Mutants in CHO Cells. Mutant and wild-type Fc$_\epsilon$RII-cDNA expression was driven by the very strong MCMV enhancer/promoter system (Fig. 1). The expression plasmids were used to stably transform $Dhfr^-$ CHO cells by selection for the $Dhfr^+$ phenotype. All the mutated Fc$_\epsilon$RII were transported to, and correctly inserted into, the cytoplasmic membrane. This was demonstrated by staining the cells with pAb raised against the C-terminal part of Fc$_\epsilon$RII expressed in *E. coli* (data not shown). The pAb detected wild-type as well as mutant Fc$_\epsilon$RII but did not stain control cells transformed with plasmid Δ1–298. Expression of mutant Fc$_\epsilon$RII at the cell surface was estimated by visually comparing the intensity of cell staining with anti-Fc$_\epsilon$RII antibodies. The intensity of cell surface staining correlates with the expression of mutant Fc$_\epsilon$RII detected by immunoblotting (see below). Staining was generally weaker for C-terminal mutants than for internal deletion mutants. pAb do not provide a good means to estimate the amount of mutant Fc$_\epsilon$RII produced by the cells, since removal of epitopes and weak expression cannot be distinguished. All internal deletion mutants were present on the cytoplasmic membrane at about the wild-type level, as estimated by

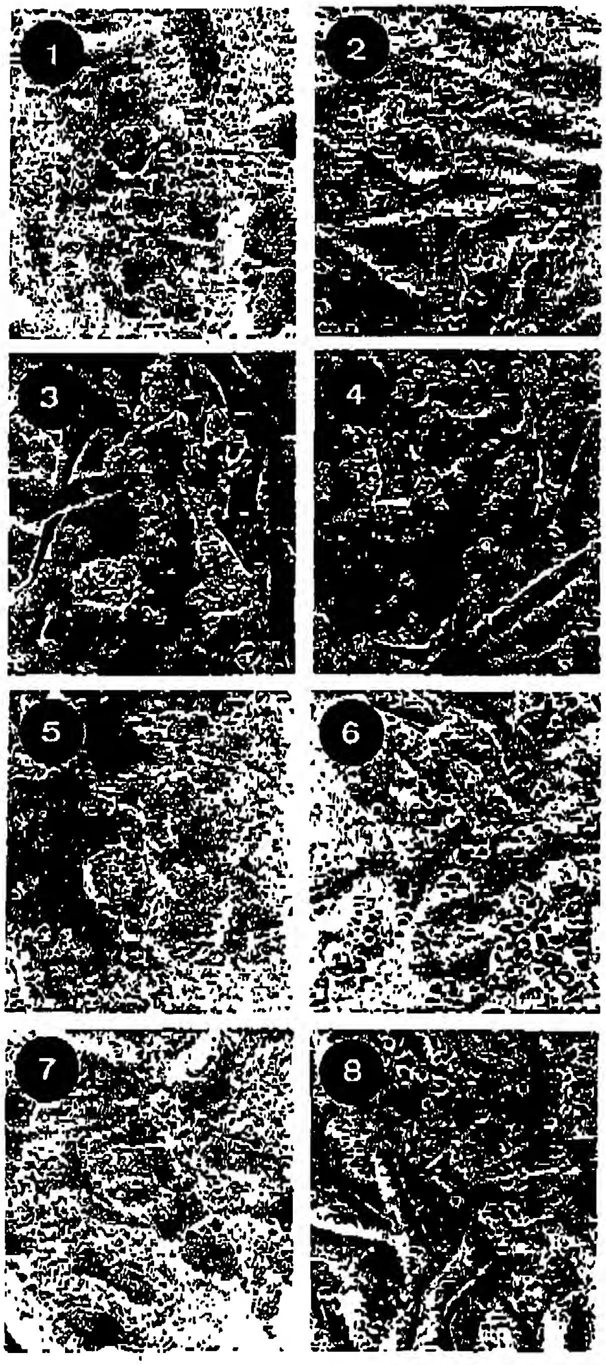

FIG. 3. IgE binding of Fc$_\epsilon$RII mutants. CHO cells stably transformed with wild-type or mutant Fc$_\epsilon$RII-cDNAs were analyzed by IgE-rosette formation with IgE-coated latex beads. Only those mutants delimiting the breakpoints of IgE-binding activity are shown. (*1*) Wild type. (2) Control Δ1–298. (*3*) Mutant X287. (*4*) Mutant X281. (*5*) Mutant Δ134–163. (*6*) Mutant Δ134–160. (*7*) Double mutant Δ134–160/X288. (*8*) Double mutant Δ134–160/X288 preincubated with IgE (1 mg/ml).

staining of the cells with mAb3-5, which recognizes a C-terminal epitope (see below). Unfortunately, there is no mAb available that recognizes all mutant Fc$_{\epsilon}$RII. Therefore, we cannot exactly determine, in the case of the C-terminal mutants, the relative amount of mutant Fc$_{\epsilon}$RII expressed at the cell surface.

Mapping the Region of Fc$_{\epsilon}$RII Essential for IgE Binding. Rosetting with immunoglobulin-coated erythrocytes (or beads) is a standard method to demonstrate expression of low-affinity Fc receptors. In our experiments, the specificity of IgE rosettes was checked by inhibition of rosette formation with pAb (data not shown) and IgE (Fig. 3). The results of the rosetting experiments are shown in Fig. 3 and summarized at the top of Fig. 4. They indicate that the C-terminal 34 residues, including Cys-288, can be removed (mutant X287) without an effect on IgE-rosette formation. Further deletion of 6 amino acid residues, which includes removal of a second cysteine at position 282 (mutant X281), impaired IgE binding. Staining of cells with pAb is about equal for these two mutants (data not shown). We therefore conclude that lack of IgE-rosette formation in mutant X281 is not due to a significant reduction of mutant Fc$_{\epsilon}$RII expression at the cell surface but represents a real functional inactivation. Internal deletion mutants lost IgE-binding activity when the deletion progressed through Cys-160 (mutant Δ134–163).

It could be argued that the alterations introduced within the IgE-BF region influence binding of IgE by perturbing the overall Fc$_{\epsilon}$RII structure. This is unlikely, since it is known that the soluble IgE-BF retains the site for binding of IgE (and also mAb, see below) (16, 18). Hence, our mutational analysis indicates that the Fc$_{\epsilon}$RII region between residues 160 and 287 is sufficient for IgE binding.

Epitope Mapping of anti-Fc$_{\epsilon}$RII mAb. Extracts prepared from cells expressing wild-type and mutant Fc$_{\epsilon}$RII were analyzed on immunoblots with pAb and mAb (Fig. 4). All the mutant proteins were recognized by pAb. As expected, progressive deletion resulted in faster mobility of mutant proteins on NaDodSO$_4$/PAGE under reducing conditions. However, under nonreducing conditions, mutants that had cysteine residues removed behaved anomalously. C-terminal deletion mutants (Fig. 4*A*) shifted to higher molecular weights when Cys-288 was removed (mutant X287). The same effect was seen with internal deletion mutants (Fig. 4*B*) when the first cysteine, Cys-160, was removed (mutant Δ134–163). This behavior on NaDodSO$_4$/PAGE indicates that cysteines 160 and 288 are engaged in disulfide bridges and that their deletion unfolds Fc$_{\epsilon}$RII.

Epitope mapping of the mAb was done under nonreducing conditions because reduced wild-type and mutant Fc$_{\epsilon}$RII proteins are not recognized by the mAb tested. MHM6 is an agonistic mAb delivering to B cells a signal indistinguishable from the effects of low molecular weight B-cell growth factor (BCGF) (5, 11, 12). All other mAb investigated are known to inhibit IgE binding to Fc$_{\epsilon}$RII (8, 28); mAb135, -176, -168, and -64 are known to belong to different epitope families (G. Delespesse, personal communication). Additionally, F(ab')$_2$ fragments of mAb135 (9) and mAb25 (10) suppress the IgE synthesis induced by interleukin 4, as well as the ongoing production of IgE by B cells isolated from atopic donors. As shown in Fig. 4, all mAb except mAb3-5 recognize the region delimited by cysteines 163 and 288. In contrast to IgE, the binding of these mAb depends on Cys-288 but not on Cys-160. mAb3-5 is an exception, since it binds the reduced Fc$_{\epsilon}$RII and inhibits IgE binding only marginally (30). Its inability to bind mutant X301 indicates that mAb3-5 recognizes the C-terminal 20 amino acid residues of Fc$_{\epsilon}$RII. Epitope mapping on immunoblots was confirmed by direct staining of cells expressing mutant Fc$_{\epsilon}$RII (data not shown).

Expression of a Minidomain That Binds Both IgE and mAb. In summary, our results establish that the amino acid sequence between positions 160 and 288 is critical for binding both IgE and anti-Fc$_{\epsilon}$RII mAb (except mAb3-5). We there-

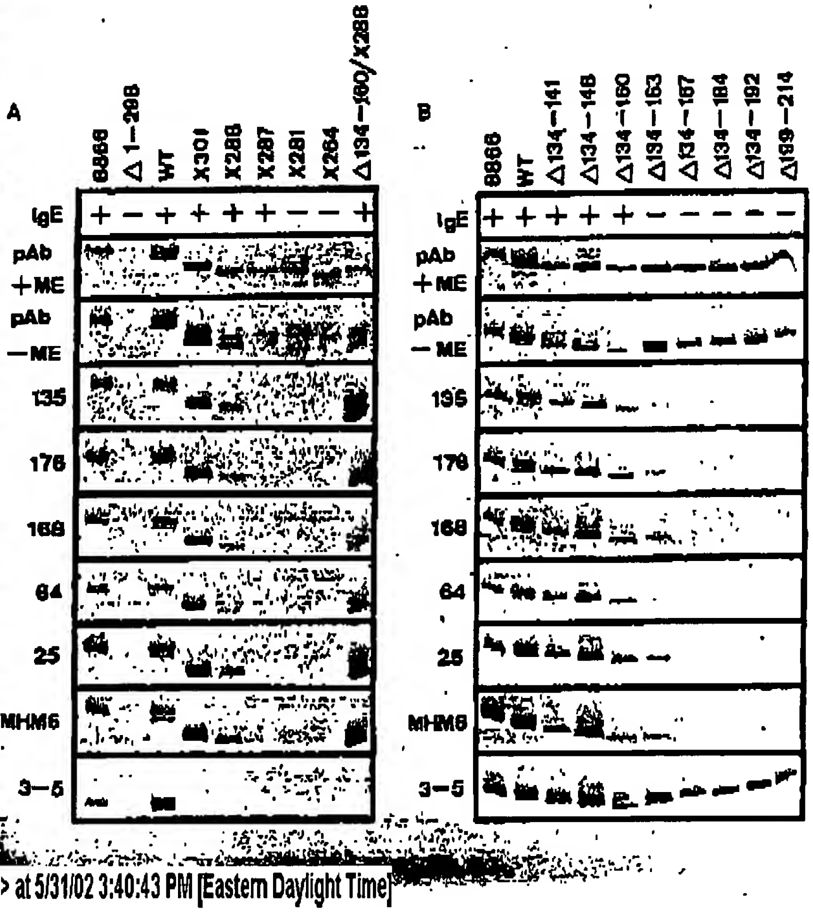

FIG. 4. Epitope mapping of anti-Fc$_{\epsilon}$RII mAb by immunoblot analysis. Solubilized cellular extracts were prepared from stably transformed CHO cells producing wild-type (WT) or mutant Fc$_{\epsilon}$RII. Additionally, Fc$_{\epsilon}$RII obtained from the B-lymphoblastoid cell line RPMI 8866 (8866) is shown. The top row of the figure indicates which of the Fc$_{\epsilon}$RII mutants do (+) or do not (−) form IgE rosettes as shown in Fig. 3. Immunoblot analysis is shown for pAb, mAb135, mAb176, mAb168, mAb64, mAb25, MHM6, and mAb3-5. For immunoblots with pAb, proteins were separated by reducing (+ME) and nonreducing (−ME) NaDodSO$_4$/12% PAGE. For immunoblots with mAb, electrophoresis was carried out under nonreducing conditions. (*A*) C-terminal deletion mutants. (*B*) Internal deletion mutants.

fore constructed mutant Δ134–160/X288, which encompasses exactly this region. As expected, the domain expressed in this mutant Fc$_\epsilon$RII is sufficient both for IgE binding (Fig. 3) and for binding all strongly inhibitory mAb and the agonistic mAb MHM6 (Fig. 4*A*).

DISCUSSION

The Fc$_\epsilon$RII, in contrast to other Fc receptors, has not evolved as a member of the immunoglobulin gene superfamily but shows substantial homology with several animal lectins (21, 22). The functional analysis of deletion mutants presented here delimits the IgE-binding domain of Fc$_\epsilon$RII to a region of 128 amino acids (residues 160–287). Strikingly, the sequence homology between Fc$_\epsilon$RII (residues 163–282) and the carbohydrate-binding domain of lectins described previously is confined to the IgE-binding domain experimentally defined in this study. The core region of homology, which includes 4 perfectly conserved cysteines, is distributed over a region of 92 amino acids comprising residues 191–282 of Fc$_\epsilon$RII (21, 22). This region of homology has all the features characteristic of C-type lectins, which are known to bind carbohydrates in a Ca^{2+}-dependent manner and to have conserved intramolecular disulfide bridges (35). In several lectins, such as the asialoglycoprotein receptors, the homology extends 30 residues further toward the N terminus and includes 2 additional conserved cysteines (21, 22). The conservation of the cysteines suggests that Fc$_\epsilon$RII and lectins have a similar folding of their homology domain. Our results indicate that the correct folding of the Fc$_\epsilon$RII homology domain is critical for function, since deletion of the cysteines has a deleterious effect on IgE binding.

Recently, Vercelli *et al.* (36) mapped the Fc$_\epsilon$RII binding site on human IgE to a motif in the C$_{\epsilon 3}$ constant-region domain that is formed on dimerization of one or both of the flanking C$_{\epsilon 2}$ and C$_{\epsilon 4}$ domains. Intriguingly, their results with enzymatically deglycosylated IgE indicated that carbohydrates are not necessary for recognition of IgE by Fc$_\epsilon$RII. It is evident that although IgE is a heavily glycosylated immunoglobulin and the binding domain of Fc$_\epsilon$RII is the lectin module, a non-lectin-type interaction takes place. Thus, the ancestral lectin module, conceived as a stable structural domain held together by disulfide bridges between conserved cysteine residues, has evolved in Fc$_\epsilon$RII to recognize the protein moiety of IgE. This unexpected finding is of interest in the light of the recent discovery in the hematopoietic system of a family of adhesion receptors containing a lectin-like domain (24). It is conceivable that lymphocyte migration is not exclusively controlled through carbohydrate-dependent recognition processes as has been proposed, but that the lectin-like domain of these adhesion receptors also displays a non-lectin activity. Conversely, it is also possible that the role of Fc$_\epsilon$RII in cell adhesion could be the result of a lectin function. It has already been proposed that Fc$_\epsilon$RII has two binding sites, one for IgE and one for carbohydrates (37). Our result would then indicate that both ligands bind within the same structural domain, raising the possibility of regulation by competitive or allosteric mechanisms.

It has been suggested that Fc$_\epsilon$RII is the receptor for low molecular weight BCGF (5, 11–13). MHM6, an anti-Fc$_\epsilon$RII mAb, interferes with BCGF uptake onto cells. This antibody also triggers an effect similar to BCGF, possibly by enhancing the processing of Fc$_\epsilon$RII into growth-promoting cleavage products (5). Further, it has been demonstrated that the growth-promoting effect of MHM6 is epitope-restricted and that Fab fragments of this mAb are also agonists of BCGF activity. This, together with our present finding that the MHM6 epitope maps to the lectin domain, suggests that this domain is the target for triggering B-cell growth. Therefore, the lectin-like domain may represent a focus for interaction between general B-cell growth and isotype-specific processes, linking together these two so far seemingly unrelated B-cell regulatory pathways and providing a means for regulation at both levels.

We are grateful to H. Rink for providing synthetic oligonucleotides; to Dr. A. Schmitz for expression of IgE-BF in *E. coli*; to Dr. E. Kilchherr for purification of recombinant IgE-BF; to Drs. J. Banchereau, G. Delespesse, J. Gordon, and T. Kishimoto for gifts of mAb; and to Drs. N. Hardman, G. Bilbe, and U. Suter for critically reading the manuscript.

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